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Johnston, M. C., & Scott, C. J. (2018). Antibody conjugated nanoparticles as a novel form of antibody drug conjugate chemotherapy. *Drug Discovery Today: Technologies*, *30*, 63-69. https://doi.org/10.1016/j.ddtec.2018.10.003

Published in:
Drug Discovery Today: Technologies

Document Version:
Peer reviewed version

Title

Antibody conjugated nanoparticles as a novel form of antibody drug conjugate chemotherapy

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Abstract

Antibody conjugated nanoparticles (ACNPs) represent a novel strategy for the development of therapies exploiting antibodies to augment the delivery of chemotherapy payloads. Following in the footsteps of the success of antibody drug conjugates (ADCs), ACNPs are only now reaching clinical evaluation. In this review we discuss the success of ADCs and explore the opportunities ACNPs offer, such as broad chemotherapy payload selection, high drug to antibody ratios and the ability to finely tailor drug release in comparison to ADCs. The ability of ACNPs to elicit increased avidity due to multivalent effects and the potential to use these modular platforms in immunotherapeutic approaches is also explored. Through addressing challenges that still remain in bringing these complex formulations to the clinic, ACNPs hold obvious potential for the treatment of a wide range of cancers and other diseases where selective targeting of drug agents is essential.

Keywords

Antibody drug conjugate; antibody conjugated nanoparticle; drug to antibody ratio; nanomedicine

Abbreviations

Antibody drug conjugate (ADC), antibody conjugated nanoparticle (ACNP), drug to antibody ratio (DAR), death receptor 5 (DR5), Enhanced Permeability and Retention (EPR), Contract Manufacturing Organisation (CMO)

Acknowledgements

This work was partially funded through a US-Ireland R&D Partnership grant awarded by HSCNI (STL/5010/14, MRC grant MC_PC_15013)

1. Introduction

The targeted delivery of chemotherapy to tumours has been a major focus in cancer research since the early 20th century [1]. Antibody drug conjugates (ADCs) are one particular method of enhancing targeting of a drug to the tumour site. Modern ADCs are monoclonal antibodies with highly potent drug molecules covalently linked to them. These 'Trojan horse' therapies are designed to target a tumour-specific receptor and be internalised where they are metabolised in the lysosome, releasing anywhere between 1 to 8 drug molecules which subsequently can elicit their cytotoxic mechanism(s) of action.

ADCs were first evaluated in the late 1950s using antibodies which targeted leukaemia cells and had the anti-cancer drug methotrexate conjugated [2]. The first in human clinical trial involving an ADC was reported in 1983 with encouraging results [3]. However, development of antibodies and ADC drugs stagnated, and it took innovations such as hybridoma fusion and humanisation to overcome the issues of single antibody species production and immunogenicity respectively; opening a pathway for the development of antibody-based drugs.

Currently there are four ADCs that have reached the market. The first to be approved in 2000 was gemtuzumab ozogamicin (Mylotarg®), which was given accelerated approval by the FDA for treatment of acute myeloid leukaemia (AML). Mylotarg® targeted CD33 and carried a DNA fragmenting payload (calicheamicin). However, it was withdrawn from the market after disappointing results from a post-approval trial, becoming the first formulation to be given accelerated approval and then subsequently withdrawn. The disappointing performance of Mylotarg® somewhat subdued industry interest in ADCs, but in 2011 and 2013 respectively, Brentuximab vedotin (Adcetris®, which targets CD30 positive lymphoma) and trastuzumab emtansine (Kadcyla®, which targets HER2 positive breast cancer) were approved; both using anti-tubulin payloads. In 2017 Besponsa® (targeting CD22 positive leukaemias) became the most recent ADC to be approved and like Mylotarg®, uses the

DNA targeting calicheamicin payload. At this time there are roughly 60 ADCs undergoing clinical trials for various cancers [4].

Another technology with potential in drug delivery are nanoparticle formulations (nanomedicine). Nanomedicine has been much heralded as a formulation-based approach to enhance the bioavailability of drug substances [5,6]. Indeed, non-targeted nanoparticles containing current chemotherapies have now reached the market. They are generally liposomes, polymeric or metal nanoparticles. One of the key clinical attributes to these formulations is their ability to reduce the toxicity profiles of the cargo chemotherapeutic thus enhancing the therapeutic window for that agent [7,8]. An exemplar of this is Doxil®, which is liposomal preparation of doxorubicin and has been on the market for over 20 years [9]. More recently, liposomal irinotecan (Onivyde®) was approved for the treatment of advanced pancreatic cancer [7].

Antibody conjugated nanoparticles (ACNPs) represent a relatively new approach that builds on the success and potential of both ADCs and nanotechnology. Conceptually ACNPs are similar to ADCs in that the antibodies can be used to specifically target diseased cells, thus delivering encapsulated cargo drug (Figure 1&2). The first targeted nanoparticles appeared in the literature in 1980 and the first to enter clinical trials was in 2011 [10–14]. Herein, we discuss the current status of the concept, its benefits and current bottlenecks.

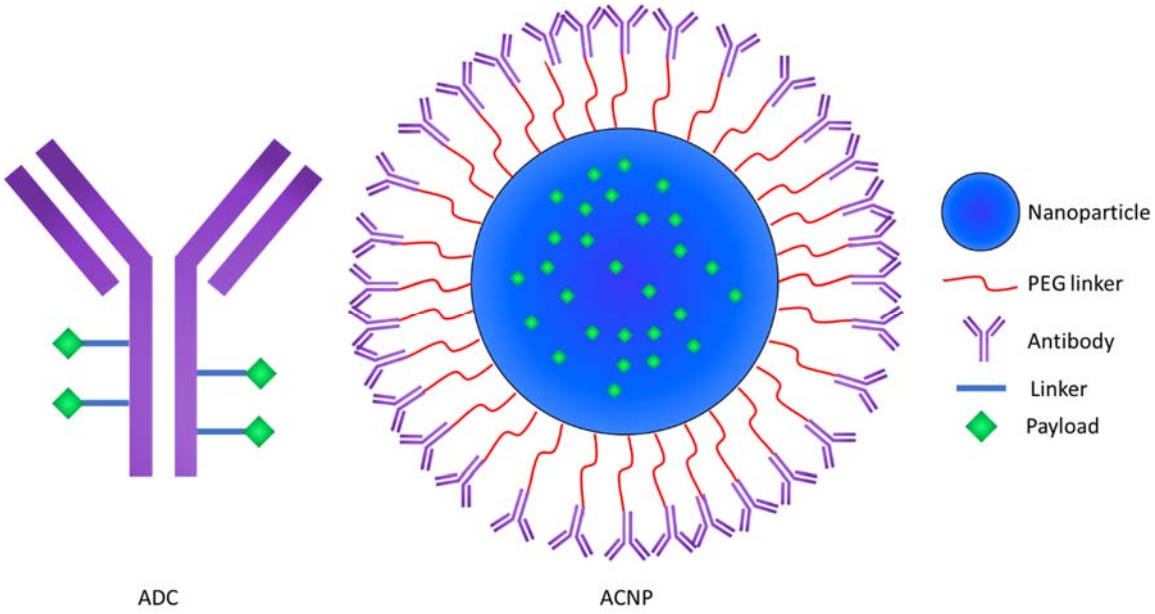

Figure 1. Schematic representation highlighting the conceptual structure of an ADC and ACNP

2. Advantages of ACNPs in chemotherapy delivery

2.1 Payload selection

A range of chemotherapies have been evaluated for encapsulation in nanoparticle systems. Whilst there are many various forms of nanoparticles, from liposomes to dendrimers, the choice of nanoparticle carrier is usually dependent on the nature and solubility of the cargo drug to ensure a high drug entrapment. For example, polymeric nanoparticles consisting of commonly employed poly-lactide based polymers are frequently used for the entrapment of hydrophobic agents, whereas liposomes can entrap hydrophobic or hydrophilic drugs in either the phospholipid bilayer of the liposome or its aqueous lumen respectively [15,16]. The combination of chemotherapies in nanoparticles has also been explored. This has advantages as it offers the opportunity to overcome pharmacokinetic differences in drug agents to ensure they are delivered at the disease site in the required proportions/ratios to provide synergistic therapeutic effects [17].

The diversity of drug agents that can be incorporated into ACNPs offers further development opportunities than can be afforded with standard ADC technologies. Early ADCs used existing standard of care chemotherapies such as methotrexate as their payload and suffered from a lack of potency as a result. Furthermore, it was found that the early linkers used had the potential to reduce payload potency [18–20]. It was soon realised that the payload conjugated to the antibody required much higher potency in order to achieve efficacy. This is because considerably fewer drug molecules were internalised (via receptor mediated endocytosis, which becomes a efficacy-limiting factor) into the target cell when compared to standard chemotherapy [21]. However, with enhanced potency, increased toxicity can be a side effect as was observed with the DNA binding calicheamicin cargo in Mylotarg®, and has driven the need for high potency payloads that are more selective towards actively dividing cancerous cells such as the anti-tubulin agents in Adcetris® and Kadcyla® [22]. Importantly, no direct drug linker is required with ACNPs and therefore this avoids disruption of the payload potency (Figure 1) [23].

2.2 Drug to antibody ratio (DAR)

Drug to antibody ratio is an important factor influencing effectiveness of ADCs. It is thought that the optimal DAR for an ADC is approximately only 4:1 for the optimal balance between cytotoxicity and acceptable pharmacokinetic profiles [24,25]. It is also important that the DAR is homogenous throughout the formulation population [26,27]. ACNPs on the other hand can potentially offer DARs over 100 and consequently when the rate limiting step for drug uptake is receptor copy number on the cell surface, ACNPs may offer an approach to ensure internalisation of much higher concentrations of drug [28]. This higher drug targeting capacity also means that drugs with lower potency than auristatins or mertansines may be successfully employed such as camptothecin derivatives [7].

2.3 Drug release

With both ADC and ACNP technologies, the selective release of the chemotherapy at the disease site is of paramount importance. In this case of ADCs, this is primarily due to the potential for side effects if healthy tissues are exposed to the highly potent payload. To prevent side effects, ADCs are mostly designed so the drug will only be released upon internalisation into the target cell. This often requires complex linker chemistry, using either cleavable or non-cleavable linkers. Cleavable linkers are usually cleaved in response to a change in the physical environment such as the lowering of pH in the lysosome or presence of a lysosomal cathepsin-labile motif. With non-cleavable linkers, release of the payload is dependent on the general degradation and metabolism of the ADC in the endo-lysosomal lumen [29].

In ACNPs, as the linker is used to conjugate the antibody to the polymer or lipid, the release of the drug is independent of this. Drug release in these formulations is therefore simply a consequence of both drug diffusion and particle degradation. Whilst almost impossible to prevent some leaching of the drug from nanoparticles unless covalently attached, the use of lower potency chemotherapies as described earlier may mean an acceptable premature release can be tolerated [30]. A strategy that has been explored to maintain drug inside polymeric nanoparticles until uptake, is the use of ion-pairing which serves to hold the drug within the nanoparticle longer through electrostatic interactions, limiting early release and providing a longer therapeutic window once at the disease site [30].

2.4 Multivalency effects

The augmenting of receptor clustering and downstream signal transduction, mimicking natural ligand binding is an approach that holds much therapeutic potential, either as an independent agent or in combination with other drugs [31–34]. In the case of ACNPs, antibody conjugation to the surface of the nanoparticle allows for the formulation to exhibit multivalency and can therefore induce hyperclustering of receptors. In the case of death

receptor 5 (DR5), monovalent targeting antibodies did not show an adequate survival benefit to warrant their use in the clinic. The requirement of Fcγ receptor activation is thought to have hindered their efficacy in clinical trials [35–37]. Encouragingly, it has been shown that conjugating an anti-DR5 antibody to the surface of a nanoparticle circumvents the need for Fcγ activation and can induce apoptosis in vitro and in vivo [38]. This could potentially offer benefit in the clinic, with the antibody functioning not only as a targeting moiety but also provides therapeutic effects itself. This avidity effect has also been exploited with nanoparticles with other targeting ligands such as carbohydrates [32,39–41].

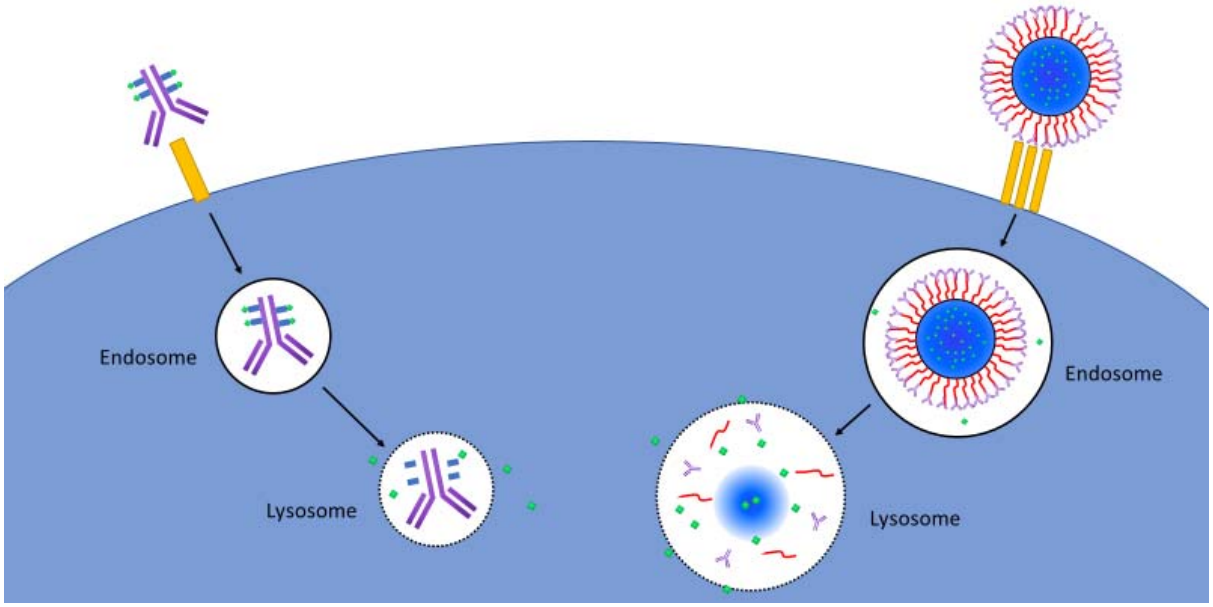

Figure 2. Schematic representation of ADC and ACNP internalisation, breakdown and drug release. (Antibodies illustrated at different scales)

3. Immunotherapy

Both passively and actively targeted nanoparticles are now being extensively researched as potential immunotherapies. They are advantageous as immunotherapies tend to require delivery of multiple payloads such as a cancer associated antigen and an adjuvant. Nanoparticles are more suited to the loading of multiple payloads due to the higher DAR they possess in comparison to ADCs.

One strategy is the targeting of dendritic cells to take advantage their antigen presenting properties. Dendritic cells can then recruit T cells against tumour specific antigen encouraging their maturation into cytotoxic T cells resulting in tumour killing. Passive targeting nanoparticles have been used to deliver both tumour-specific antigens and adjuvants to dendritic cells. It has been demonstrated that altering particle size can increase dendritic cell internalisation when administered subcutaneously [42,43]. The use of active targeting has been shown to improve delivery to antigen presenting cells resulting in improved production of cytotoxic T cells and reduced tumour growth in vivo [44]. The dendritic cell targeting formulation Lipovaxin-MM has passed a phase 1 dose escalation study [45].

An alternative approach to targeting antigen presenting cells to stimulate T cells is to target T cells directly. ACNPs have also been used to target T cells directly in mouse models which ultimately showed reduced tumour growth and increased survival [46]. While ACNPs have not yet reached clinical trial for their potential use in immunotherapy, the data so far is promising.

4. Barriers facing ACNPs pathway to the clinic

There are only a limited number of ACNPs that have reached clinical trials which are summarised in Table 1. None have yet reached phase 3. While the passively targeted nanoparticle formulations that have reached the market generally cause a more favourable pharmacokinetic profile than their free payload, it does require careful consideration of aspects of nanoparticle size, shape and surface charge. A survey of 117 nanomedicine publications presenting quantitative biodistribution data in cancer models revealed that only a median average of 0.7% of nanoparticles reach the tumour site *in vivo* [47]. While it was acknowledged that the formulations on the market or in clinical trial were much higher than this figure, further development is needed to increase the median average across the board.

The nanomedicine field had over relied on the enhanced permeability and retention (EPR) effect to increase delivery to the tumour; the ability of nanomedicines to seep into tumours due to tumour neovasculature leakiness and reduced interstitial pressure. The FDA approved imaging formulation Ferumoxytol® has been shown to be predictive of nanoparticle accumulation *in vivo* which could one day give clinicians a useful tool to predict how beneficial nanomedicine could be for a specific patient – a personalised or precision medicine approach [48]. In an effort to increase tumour delivery, methods are now emerging to enhance 'permeability' of the tumour rendering it more susceptible to nanoparticle deposition by selectively targeting the integrity of the tumour specific neovasculature. This has proven to be successful *in vivo* [49,50]. Thus, using approaches like this to overcome reliance on EPR will be essential in order to realise the potential of active targeting on ACNPs. By using the antibody to not only target the tumour but also elicit independent therapeutic effects can only enhance the opportunities provided by the technology [47].

Another key bottleneck at this time is the developability and manufacturability of ACNPs. Routes to synthesis and access to CMOs specialising in the large-scale cGMP manufacturing of ACNPs is an issue that will need to be overcome in order for the technology to become more widely adopted. However, these are issues that have been faced previously with biologics manufacture and with ADC manufacture itself [51,52]. Therefore, if therapeutic efficacy of the formulations can be clearly demonstrated, the investment needed by large pharmaceutical organisations in manufacturing to overcome this 'valley of translational death' will be de-risked and made more compelling.

Table 1. Antibody targeted nanoparticle formulations that have gone through or are currently undergoing clinical trial adapted from *Richards et al.* and *van der Meel et al.* [53,54]

Name	**Target**	**Ligand**	**Type**	**Payload**	**Indication**	**Phase**
SGT-53	Transferrin receptor	Anti-transferrin receptor ScFv	Lipid	P53 DNA	Recurrent Glioblastoma	II
SGT-94	Transferrin receptor	Anti-transferrin receptor ScFv	Lipid	RB94 DNA	Solid tumours	I
C225-ILS-Dox	EGFR	Cetuximab Fab	Lipid	Doxorubicin	High-grade gliomas	I
MM-302	HER2	Anti-HER scFv	Lipid	Doxorubicin	Breast cancer	II
MM-310	Ephrin receptor A2	Anti-EphA2 scFv	Lipid	Docetaxel	Solid tumours	I
MCC-465	Uncharacterised (GAH)	Anti-GAH $F(ab')_2$	Lipid	Doxorubicin	Metastatic stomach cancer	I

Lipovaxin-MM	Dendritic cell CD209	dAb	Lipid	Melanoma antigens + IFNγ	Melanoma	I
Erbitux-$EDVs_{pac}$	EGFR	Bispecific monoclonal antibody (mAb)	Bacterially derived mini-cell	Paclitaxel	Solid tumours	II

5. Conclusion

The best way to use the targeting abilities of antibodies may never be agreed. ADCs have proven their ability to deliver cytotoxic payloads to tumours and are currently the most beneficial targeted, drug conjugated therapy for patients. ACNPs however may allow for existing chemotherapies to be made available in nanomedicinal preparations. Drug release from nanoparticles can be more finely controlled with a range of nanoparticle materials and co-excipients to choose from. ACNPs may provide benefit over ADCs when targeting receptors where receptor agonism can derive additional therapeutic effects. Furthermore, in this age of immunotherapy, the ability to use ACNPs to augment immune responses to tumours also hold much promise. With strategies to enhance the ability of these agents to reach tumours to facilitate active targeting, combined with improved uniform manufacturability from improved conjugation chemistries [55], it is anticipated that there will be an increase in the interest of these agents for clinical evaluation.

Conflict of interest

Michael Johnston declares no conflict of interest

Christopher Scott is a consultant to Fusion Antibodies Ltd.

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